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OPTIMIZATION OF HPLC METHOD FOR CONTROL OF PROPRANOLOL HYDROCHLORIDE IMPURITIES IN LIQUID MEDICINE FOR ORAL USE IN PEDIATRICS

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Propranolol hydrochloride is approved in Ukraine as one of the drugs for the treatment of infantile hemangiomas in pediatrics, however, there are no industrially produced drugs, which necessitates the development and small-scale production. To ensure their proper quality, it is important to implement high-precision analytical control methods, to determine accompanying impurities that may affect the safety and effectiveness of therapy in children.
***Aim**. To optimize the liquid chromatography method for determining impurities of the active pharmaceutical ingredient in the drug "Propranolol hydrochloride, 4.28 mg/ml, oral solution", manufactured by pharmacy No. 2 CHEMOTEKA of the Pharmaceutical Department of PE "Infusia", intended for use in pediatrics.*
***Materials and methods.** The study was carried out as part of the development of the medicinal product "Propranolol hydrochloride, 4.28 mg/ml, solution for oral administration" (pharmacy No. 2 CHEMOTEKA, PE "Infusia"). Impurities were controlled by liquid chromatography according to the US Pharmacopoeia method "Propranolol hydrochloride for injection". Method verification was carried out considering the requirements of international standards and the SPhU.*
***Results**. The proposed method for determining the content of related impurities is characterized by high specificity, linearity in the range of 0.5–1.6 mg/ml, accuracy, precision, confirmed reproducibility and reporting limit at the level of no more than 0.1%. Additionally, the stability of solutions for 24 hours has been established, which ensures the convenience of its use in routine pharmaceutical analysis.*
***Conclusions**. The optimized liquid chromatography technique provides reliable determination of impurities in the medicinal product "Propranolol hydrochloride, 4.28 mg/ml, oral solution", meets international validation requirements and can be used in routine quality control. The results obtained confirm the feasibility of its use to improve the safety and effectiveness of therapy with the study drug*
***Keywords**: verification, liquid chromatography, related substances, propranolol hydrochloride, small-scale manufacturing, infantile hemangioma*

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1. Introduction

The use of propranolol hydrochloride – (2RS)-1-[(1-methylethyl)amino]-3-(naphthalen-1-yloxy)-propan-2-ol hydrochloride, $C_{16}H_{22}ClNO_2$ Mol. wt. 295.8) in pediatric practice in the form of a solution for oral administration, primarily for the treatment of infantile hemangiomas, has become a significant advancement in pediatric pharmacotherapy [1]. Despite the proven effectiveness of the drug, the problem of the lack of standardized dosage forms for children in Ukraine and a number of other countries persists, which leads to the widespread off-label use of propranolol hydrochloride medicines [2].

Propranolol is characterized by complex metabolism and pharmacokinetic features that require the use of high-precision analytical methods to confirm the quality, stability and safety of drugs [3]. Despite the fact that the drug in the form of a syrup containing 4.28 mg/ml propranolol hydrochloride (equivalent to 3.75 mg/ml propranolol) has been approved by the U.S. Food and Drug Administration (FDA) since 2014 (the original drug is "Hemangeol" (Pierre Fabre Pharmaceuticals Inc., Paris, France) and since 2016 in Ukraine it has been approved by the Ministry of Health of Ukraine in the clinical protocol for systemic therapy of vascular anomalies in children in the form of infantile hemangiomas, industrially produced drugs and, accordingly, a monograph in the State Pharmacopoeia of Ukraine are absent [4, 5].

Despite the demand, pharmacy No. 2 CHEMOTEKA of the Pharmaceutical Department of PE "Infusia" has launched small-scale production of the medicinal product "Propranolol hydrochloride, 4.28 mg/ml, oral solution". Since the drug is used in young children, even a small number of impurities or degradation products can lead to a change in the therapeutic effect and the occurrence of side effects [6]. Therefore, the detection and control of impurities is an integral part of the quality, safety and efficacy of the medicinal product.

The British Pharmacopoeia, the State Pharmacopoeia of Ukraine and the United States Pharmacopoeia present monographs on the substance propranolol hydro-

chloride [7–9] and "Propranolol tablets" [10–12], and the United States Pharmacopoeia monograph "Propranolol hydrochloride for injection" [13] has attracted particular attention, since the application of the requirements of the injectable preparation to the oral form determines its use in pediatric practice. This has led to the relevance of the verification of the liquid chromatography method for the determination of impurities of propranolol hydrochloride in the oral dosage form.

The implementation of this task will facilitate the possibility of controlling impurities at all stages of the life cycle of the drug and will contribute to the creation of safe and effective dosage forms for children, harmonization of national standards with international requirements.

2. Research planning (methodology)

The feasibility of optimizing the pharmacopoeial liquid chromatography method is because the USP Propranolol Hydrochloride Injection monograph method is designed for a parenteral dosage form and does not take into account the presence of excipients that are used in oral dosage forms. Therefore, the optimization of the method was aimed at ensuring the specificity and reliability of the determination of impurities in a multicomponent oral dosage form for pediatric use.

The research methodology is structured as follows:

– to analyze the possibility of using the pharmacopoeial method of liquid chromatography for the determination of impurities of propranolol hydrochloride in a liquid dosage form for oral use in pediatrics, taking into account the presence of excipients, based on the requirements of the USP monograph *Propranolol Hydrochloride Injection*;

– select the optimal conditions of the chromatographic system (column, mobile phase, detection wavelength, flow rate, injection volume) taking into account the physicochemical properties of the active substance and auxiliary components of the dosage form, in particular the "Vanilla" flavouring, which may affect the specificity of the analysis;

– conduct verification studies in accordance with the requirements of USP ⟨1226⟩ Verification of compendial procedures, SPhU and PA/PH/OMCL recommendations, which provide for verification of the following parameters: suitability of the chromatographic system, specificity, linearity and range of application, accuracy, precision (convergence), limit of quantification, stability of analytical solutions over time;

– determine the criteria for acceptability of results: the content of any individual impurity – no more than 0.2%, the sum of impurities – no more than 0.5%, the reporting limit – no more than 0.1%;

– to investigate the stability of analytical solutions for 24 hours when stored in an autosampler thermostat to confirm the possibility of their use in routine quality control of medicinal products.

3. Materials and methods of the research

The object of the study was the extemporaneous drug "Propranolol hydrochloride, 4.28 mg/ml oral solution", pharmacy No. 2 CHEMOTEKA of the Pharmaceutical Department of PE ″Infusia", which is a liquid dosage form for oral use in pediatrics, containing 4.28 mg/ml of propranolol hydrochloride (equivalent to 3.75 mg of propranolol).

Experimental studies were conducted from the fourth quarter of 2024 to the third quarter of 2025. The studies were carried out on a liquid chromatograph – Agilent 1260 and 1290, equipped with UV/diode array detectors (Agilent Technologies, USA); analytical balance – Mettler Toledo MS104 (Mettler Toledo, Switzerland); Symmetry chromatographic column 250 cm in size, 4.6 mm in diameter with a particle size of 5 μm, pH meter Mettler Toledo SevenCompact 220 (Mettler Toledo, Switzerland). Sample preparation was carried out using class A measuring vessels and reagents that meet the requirements of EP/SPhU.

The method for determining impurities was based on the United States Pharmacopoeia (USP) monograph Propranolol Hydrochloride Injection (USPNF 2022 Issue 3, ver. 01/12/2022) [13].

The protocol was developed in accordance with the requirements of ⟨1226⟩ Verification of compendial procedures (USP42-NF37 2S) [14], recommendations PA/PH/OMCL (13) 82 R5 [15] and SPhU 5.3.N.2 Validation of analytical methods and tests [16]. To confirm the possibility of using the method for the test "Companion impurities" it is necessary to check the following parameters: system suitability, specificity, linearity, precision, accuracy, limit of quantification, stability of solutions over time. Since the method is pharmacopoeial, a robustness study was not required.

The research used experimental and industrial batches of the drug "Propranolol hydrochloride, 4.28 mg/ml oral solution " (p. 15042024-1, 06112023-1, Pharmacy No. 2 CHEMOTEKA of the Pharmaceutical Department of PE "Infusia", Ukraine).

As standard samples, the following were used: propranolol hydrochloride (p. 22142PRRII, IPCA Laboratories Ltd. India), (3-(Naphthalen-1-yloxy)propane-1,2-diol) (propranolol hydrochloride impurity A) (p. R4196586, UOSLAB LLC, Ukraine).

The test is performed by liquid chromatography (SPhU, European Pharmacopoeia, 2.2.29) according to the USP method "Propranolol Hydrochloride Injection" [13, 17, 18].

Solvent. Mobile phase.

Test solution. 11.7 ml of the test dosage form (equivalent to 50.076 mg of propranolol hydrochloride) is transferred to a 100.0 ml volumetric flask, dissolved in the mobile phase and the volume of the solution is brought to the mark with the same solvent (nominal concentration of propranolol hydrochloride is 0.501 mg/ml).

Reference solution (a). 10.0 mg of propranolol hydrochloride (USP RS, SPhU RS, WRS or of equivalent quality) is dissolved in the mobile phase. If necessary, ultrasound is used. The volume of the solution is brought to 100.0 ml with the mobile phase. 1.0 ml of the resulting solution is brought to 100.0 ml with the mobile phase. (nominal concentration of propranolol hydrochloride is 1 μg/ml).

Comparison solution (b). Dissolve 10.0 mg of propranolol hydrochloride impurity A (CAS No. 36112-95-5;

USP RS, SPhU RS, WRS or of equivalent quality) in the mobile phase. If necessary, use ultrasound. Dilute with the mobile phase to 100.0 mL (nominal concentration of propranolol hydrochloride impurity A: 0.1 mg/mL).

Comparison solution (c). Solution for system suitability. 50.0 mg of propranolol hydrochloride (USP RS, SPhU RS, WRS or of equivalent quality) is dissolved in the mobile phase, if necessary, using ultrasound, add 1.0 ml of reference solution (b). The volume of the solution is brought to 100.0 ml with the mobile phase. (nominal concentration of propranolol hydrochloride – 500 µg/ml, propranolol hydrochloride impurity A – 1 µg/ml).

Comparison solution (d). Solution for checking the sensitivity of the system. 5.0 ml of reference solution (a) is brought to 10.0 ml with the mobile phase (nominal concentration of propranolol hydrochloride – 0.5 µg/ml).

Mobile phase. dissolve 1.6 g of sodium dodecyl (lauryl) sulfate R and 0.3 g of tetrabutylammonium phosphate R in a mixture containing 1 ml of sulfuric acid R, 450 ml of water for chromatography R and 550 ml of acetonitrile R. Adjust the pH of the resulting solution to 3.3 with 2 M sodium hydroxide solution.

Chromatography is carried out on a liquid chromatograph equipped with a UV/diode array detector under the following conditions:

– chromatographic column 250 mm length, 4.6 mm in diameter, filled with octadecyl silyl silica gel for chromatography P with a particle size of 5 µm (e.g. Symmetry Waters, or similar for which the requirements for the suitability of the chromatographic system are met);

– detection: 292 nm;

– flow rate: 1.8 ml/min;

– injection volume 50 µl;

– chromatography time – must be at least 9 times the retention time of the main peak of propranolol.

Chromatograph reference solutions (c), (a) and (d).

The relative retention times for propranolol impurity A and propranolol are 0.6 and 1.0 respectively.

The chromatographic system is considered suitable if:

– in the chromatogram obtained from reference solution (c) the separation between the peaks of propranolol impurity A and propranolol is not less than 3.0;

– the relative standard deviation calculated for the propranolol peak obtained from 5 parallel chromatograms of reference solution (a) is not more than 5.0%;

– the signal-to-noise ratio calculated for the propranolol peak obtained in the chromatogram of the reference solution (d) is not less than 10.

Chromatograph the test solution.

The percentage content of any impurity (X_i) is calculated using the following formula

$$X_i = \frac{S_i \cdot m_0 \cdot P_0 \cdot 100.0 \cdot 1.0 \cdot 100 \cdot}{S_0 \cdot 11.7 \cdot 4.28 \cdot 100 \cdot 100.0 \cdot 100.0} = \frac{S_i \cdot m_0 \cdot P_0}{S_0 \cdot 5007.6},$$

where S_i – the area value of any degradation impurity obtained in the chromatogram of the test solution; S_0 – the average value of the peak areas of propranolol obtained in the chromatogram of the reference solution (a); m_0 – mass of the portion of propranolol hydrochloride taken to prepare reference solution (a), in milligrams; P_0 – content of propranolol hydrochloride in the sample taken for the preparation of reference solution (a), in percentage; 11,7 – aliquot of the dosage form taken to prepare the test solution, in milliliters; 4,28 – nominal content of the active substance propranolol hydrochloride in the dosage form, in milligrams per 1 milliliter.

Acceptance criteria:

– the retention time of the peak of propranolol hydrochloride in the dosage form on the chromatograms of the test solution must coincide with the retention time of the corresponding peak on the chromatogram of the reference solution (a) with an accuracy of 1.85%;

– the content of any individual degradation impurity is no more than 0.2%;

– the sum of impurities is no more than 0.5%;

– reporting threshold 0.1%.

Peaks of the blank solution and below the reporting threshold are not considered.

4. Research results

To confirm the reproducibility and practical application of the method for determining related impurities in the process of routine control, validation studies were conducted, which included an assessment of the suitability of the chromatographic system, specificity, linearity and range of application, precision, accuracy, and confirmation of the limit of quantitative determination. Additionally, in order to ensure the reliability of control, the shelf life of analytical solutions was determined. Considering that the method has a pharmacopoeial origin, conducting studies of the method's resistance to minor changes (robustness) is not mandatory, since its parameters have been standardized and confirmed at the level of pharmacopoeial requirements.

Suitability of the chromatographic system. For routine verification of the method, a number of parameters were determined to confirm the sensitivity of the system. The separation between the peaks of propranolol and its impurity A is an important indicator of the specificity of the method and ensures the correctness of the quantitative determination of impurities without mutual overlapping of signals. The relative standard deviation for the peak of propranolol meets the pharmacopoeial requirements for precision and demonstrates the stability of the system during repeated measurements. The signal-to-noise ratio also confirms the sensitivity of the method and the ability to detect even low levels of impurities.

According to the requirements of the European Pharmacopoeia and SPhU, these parameters are key in the validation of impurity control methods, as they ensure both analytical reliability and reproducibility of results in routine control.

To verify the suitability of the chromatographic system, a sufficient number of parallel chromatograms of reference solutions (c), (a) and (d) were obtained and analyzed (Table 1). The data indicate and confirm the suitability of the system.

Table 1

Results of the suitability study of the chromatographic system

No.	Parameter	Value	Result	Conclusion
1	Separation between peaks of propranolol impurity A and propranolol	at least 3.0	≥ 8.3	Corresponds
2	Relative standard deviation calculated for the propranolol peak obtained from 5 parallel chromatograms of reference solution (a)	at least 5.0%	1.63%	Corresponds
3	Signal-to-noise ratio calculated for the propranolol peak obtained in the chromatogram of the reference solution (d)	at least 10	≥114	Corresponds

Specificity. To confirm the specificity of the analytical method and the identification of analytes of the determined substances, the chromatograms obtained from the control solution, reference solutions, the test substance and solutions of individual substances of the multicomponent mixture were compared.

The chromatograms of the control (blank) solution do not have additional peaks, the retention times of which would coincide with the retention times of the target peaks of Propranolol and the identified impurity A. The retention times of the peaks of the excipients, except for the flavoring "Vanilla", do not coincide with the retention times of the target peaks of the main substance and its identified impurity. Thus, they do not interfere with the determination of related impurities in the analyzed dosage form.

Regarding the influence of the main and additional peaks of the "Vanilla" flavoring on the determination of related impurities in the dosage form, the following should be noted. On the chromatogram obtained from the "Vanilla" flavoring solution prepared at a concentration corresponding to the flavoring content in the test solution, a flavoring impurity peak is observed (RT = 2.12 min, $S_{placebo}$ = 10.8), the retention time of which coincides with the retention time of propranolol impurity A (RT = 2.12 min) on the chromatogram obtained from the test solution ($S_{i+placebo}$ = 11.8) and on the chromatogram of the reference solution (d) (RT = 2.111 min, $S_{i\,RSd}$ = 38.7).

To determine the degree of influence of the impurity of the flavoring "Vanilla" on the determination of propranolol, impurity A, the areas of the obtained peaks were compared. The interfering signal should be not more than 10% of LOQ (S_{LOQ} = 18.8).

$S_{placebo} \leq 10\%$ from LOQ;

$10.78 \leq 1.9$.

The peak of the flavoring impurity with a retention time (RT = 2.12 min) has a significant impact on the determination of propranolol impurity A. Therefore, it is optimal to subtract the area of the peak of the flavoring impurity from the area of the peak of propranolol impurity A.

Therefore, the analytical method can be considered sufficiently specific and can be used to determine impurities in the dosage form of 4.28 mg/ml propranolol hydrochloride, provided that when calculating the area of the peak of the flavoring impurity with a retention time ($RT \approx 2.11$) obtained on the chromatogram of the reference solution of the flavoring "Vanilla" is subtracted from the area of the peak with a similar retention time obtained on the chromatogram of the test solution (Fig. 1–6).

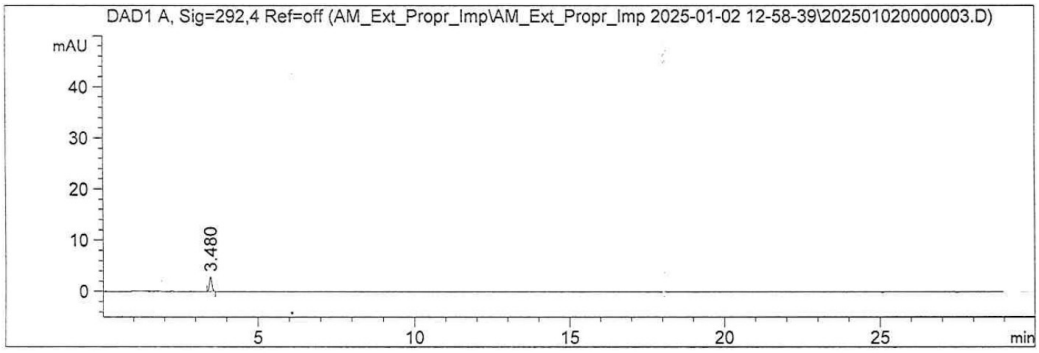


Fig. 1. Typical chromatogram of reference solution (d)

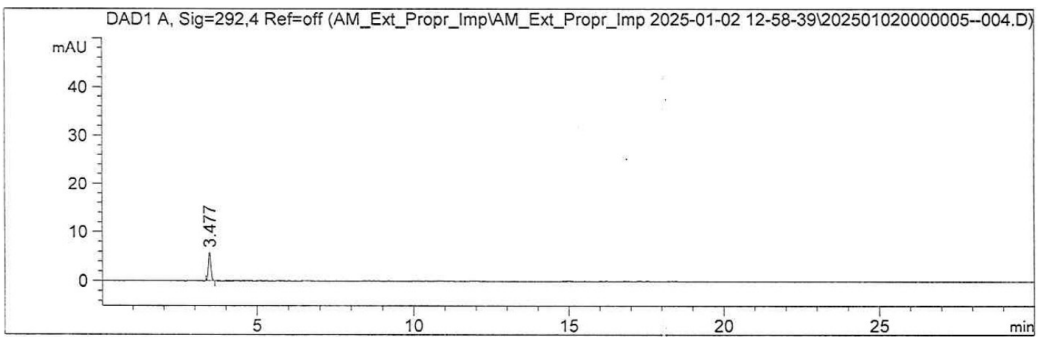


Fig. 2. Typical chromatogram of the reference solution (a)

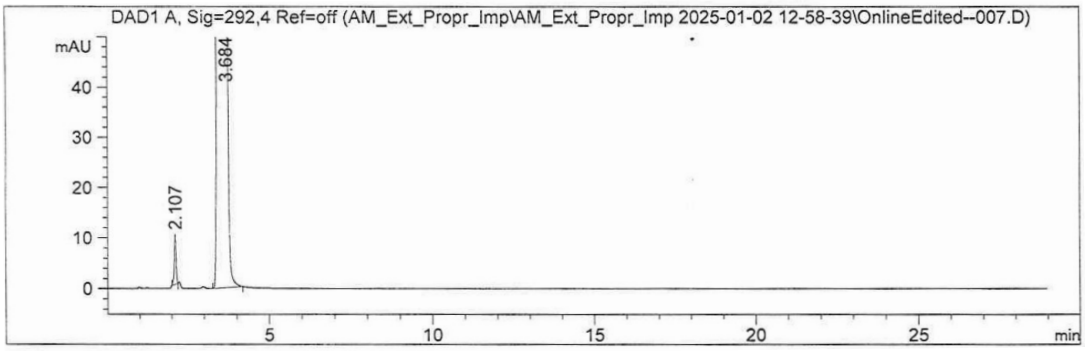


Fig. 3. Typical chromatogram of the reference solution (c)

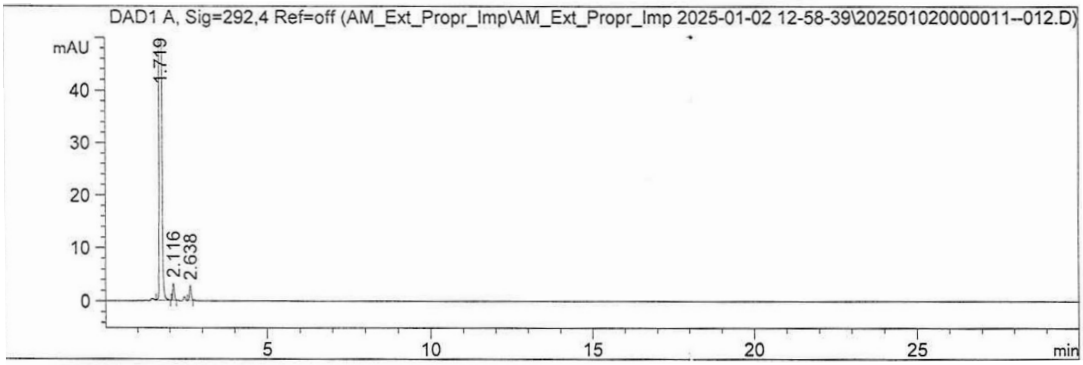


Fig. 4. Typical chromatogram of a solution of the flavoring "Vanilla"

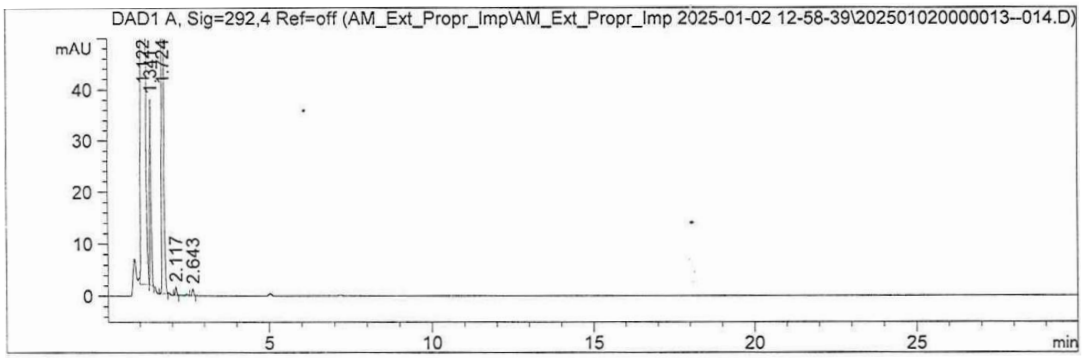


Fig. 5. Typical chromatogram of placebo solution

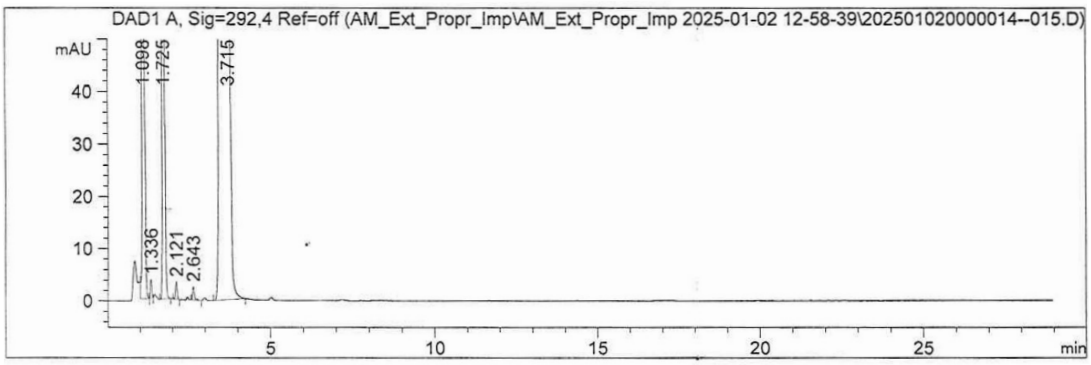


Fig. 6. Typical chromatogram of the test solution

Linearity and range of application of the method. Linearity studies for propranolol hydrochloride and propranolol impurity A were carried out in the concentration range from 0.5 μg/ml to 1.6 mg/ml (which corresponds to a content of 0.10–0.32% of the propranolol hydrochloride content in the test solution) for both analytes.

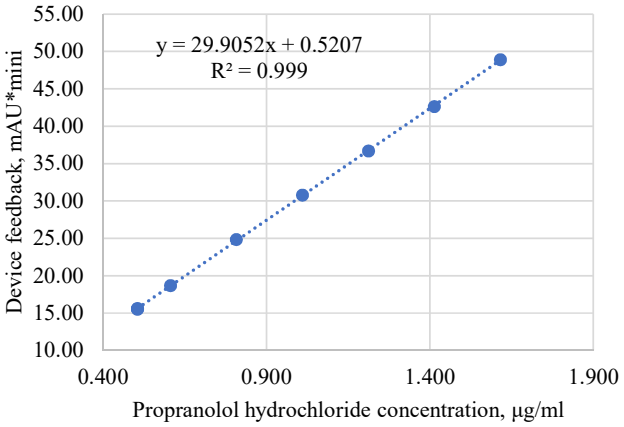
Linear regression parameters were calculated in accordance with the recommendations of SPhU 5.3.N1. Statistical analysis of the results of the chemical experiment [16]. The results of the study of the parameters of the linear regression of the analytical signal of the results are presented in Table 2.

Table 2

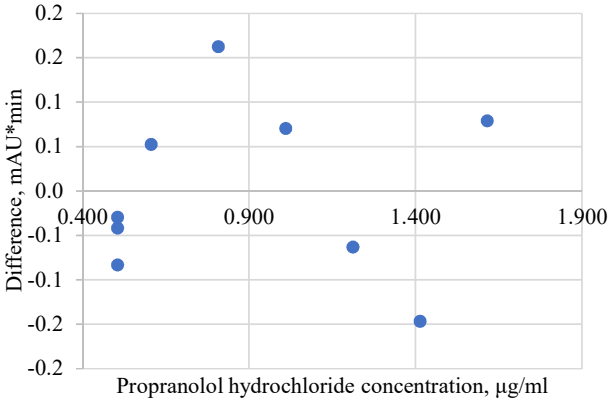
Linear regression parameters

Parameter	*a*	|*a*|, %	*b*	*r*
Criteria	N/A	≤ 5.0	N/A	≥ 0.990
Propranolol hydrochloride	0.5207	1.6523	29.9052	0.99997
Propranolol impurity A	0.7731	2.0933	35.5713	0.99998

The plots of the linear dependence of the analytical signal response on the concentration and the plots of the residues of propranolol hydrochloride and propranolol impurity A are presented in Fig. 7, 8, respectively.



a



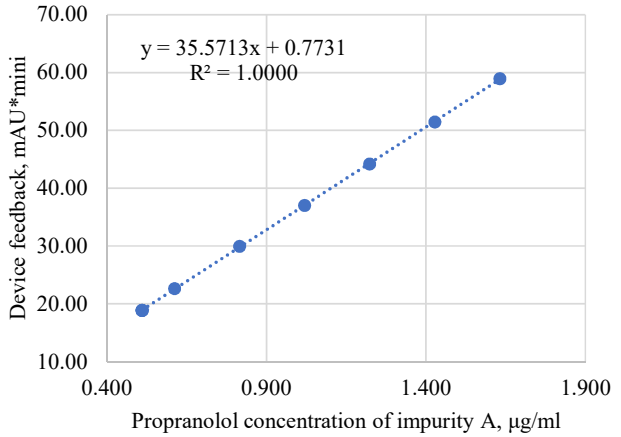
b

Fig. 7. Graph of the dependence of the device response on the concentration of propranolol hydrochloride in model solutions (determination of unspecified impurities): *a* – regression graph; *b* – residual graph

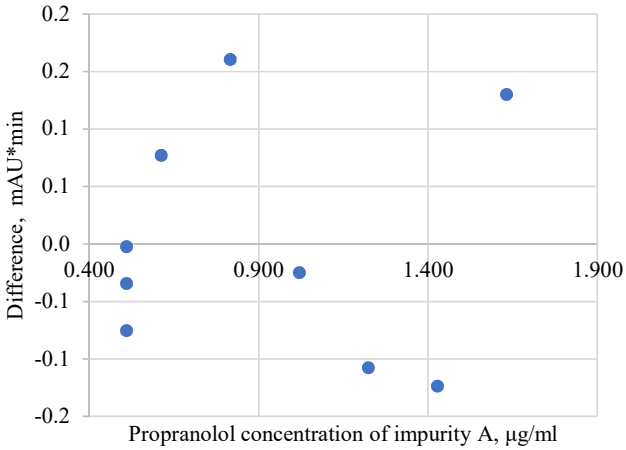
The calculated values meet the suitability criteria; the residuals are randomly scattered around 0. The linearity of the method has been proven in the specified range.

Assessment of the need to introduce conversion factors. The calculation of the conversion factor was performed using the ratio of the slope of the regression equation for propranolol hydrochloride (b_{PH}) to the slope of the regression equation for propranolol impurity A (b_{impA}) according to the formula

$$K_1 = \frac{b_{PH}}{b_{impA}} = \frac{29.9052}{35.5713} = 0.841.$$



a



b

Fig. 8. Graph of the dependence of the device response on the concentration of propranolol impurity A in model solutions: *a* – regression graph; *b* – residual graph

For calculations, the conversion factor can be omitted, since it is within the recommended range of 0.8–1.2.

Accuracy. To assess the accuracy of the method, 18 model solutions were prepared containing a known addition of impurity A in the test solution and 3 parallels of the test solution without the addition of the impurity C.

The impurity content was calculated using the formula

$$C_t = \frac{(S_i - S_{Vanilla}) \cdot C_{ref}}{S_{ref}},$$

where S_i – area of the analyte peak from the chromatogram of the model solution; $S_{Vanilla}$ – peak area of the "Vanilla" flavoring impurity, with a retention time that coincides with the retention time of propranolol impurity A from the chromatogram of the model solution; C_{ref} – concentration of the analyte in the reference solution, mg/ml; S_{ref} – peak area of the analyte of the reference solution.

The results of the calculation of impurity A are given in Table 3. The evaluation of the results is given in Table 4.

The results obtained satisfy the acceptance criteria, i.e. the correctness of the method is sufficient.

Precision. To study the precision, 6 test solutions spiked with impurity A at the standardization

level (M2-1–M2-6) were studied. The impurity content in the dosage form, the average impurity content, and the RSD between the calculated impurity content were calculated. The chromatography results and the evaluation of the results are given in Table 5.

The results obtained satisfy the acceptance criteria. The precision of the method is sufficient.

Table 7

Evaluation of the sensitivity of the method for the determination of propranolol impurity A

Solution		L1-1	L1-2	L1-3	M1-1	M1-2	M1-3	M1-4	M1-5	M1-6
Concentration, μg/mL		0.510			0.510					
Concentration, % of API		0.1018			0.1019					
S/N	Criteria	≥ 10								
	Result	314.5–488.7			398.7–571.4					
S_{Av}		18.88	18.84	18.91	28.79	28.76	28.75	28.76	28.64	28.74
RSD, %	Criteria	≤ 15.0								
	Result	0.134	0.095	0.186	0.024	0.015	0.014	0.119	0.286	0.089
Recovery, %	Criteria	80.0–120.0%								
	Result	100.237	100.018	100.406	96.108	95.924	95.867	95.932	95.306	95.838
$Recovery_{Av}$, %		100.220			95.829					
$RSD_{Recovery}$, %	Criteria	≤ 20.0								
	Result	0.194			0.285					

Table 3

Calculation of impurity A content in the dosage form

Solution	CS Vanila (*RT* = 2,098)	CS (impurity A)	M0_1	M0_2	M0_3
S_i	10.73	31.51	10.87	10.84	10.82
Aliquot of dosage form, ml			11.7	11.7	11.7
C_m, mcg/ml			0.0046	0.0038	0.0031
$C_{m\,aver}$, mcg/ml			0.0038		

Table 4

Correctness rating

Parameter		Acceptance criterion	Result
Individual extraction degree values	Min	≥ 80.0%	111.76%
	Max	≤ 120.0%	119.05%
Average extraction rate		80.0–120.0%	115.88%

Table 5

Results of chromatography of test solutions

Solution	CS	M2-1	M2-2	M2-3	M2-4	M2-5	M2-6
Average	31.51	48.34	48.38	48.35	48.40	48.36	48.33
RSD, %	1.63	0.06	0.03	0.05	0.01	0.15	0.04
X, %	n/a	0.2014	0.2016	0.2014	0.2017	0.2015	0.2013
Average, %		0.2015					
RSD, % (≤ 10.0%)		0.074					

Limit of quantification. To confirm the limit of quantification, the signal-to-noise ratio and the degree of recovery were calculated for the peak of propranolol hydrochloride and its impurity A in model solutions L1-1–L1-3 and M1-1–M1-6. The results are given in Tables 6, 7.

Table 6

Evaluation of the sensitivity of the method for determining unspecified impurities

Solution		L1-1	L1-2	L1-3
Concentration, μg/mL		0.505		
Concentration, % of API		0.101		
S/N	Criteria	≥ 10		
	Result	177.8–276.8		
S_{Av}		15.59	15.54	15.58
RSD, %	Criteria	≤ 15.0		
	Result	0.119	0.153	0.144
Recovery, %	Criteria	80.0–120.0%		
	Result	98.771	98.431	98.694

The sensitivity of impurity A and unspecified impurities was confirmed at 0.10%.

Stability of solutions. The stability of analytical solutions was investigated by storing them in vials in an autosampler thermostat for 24 hours. To assess this parameter, both the reference solution and the spiked solution M2 were analyzed. The test results showed that both solutions remained stable for the specified time, which confirms the possibility of their use in routine control conditions without the risk of loss of analytical accuracy or reproducibility.

5. Discussion of research results

In the absence of a registered medicinal product of propranolol hydrochloride suitable for children, in Ukraine and many countries of the world, the dosage form is modified extemporaneously by grinding the medicinal product in the form of tablets (with an active ingredient content of 10 mg or 40 mg) and preparing a powder dosage form with the addition of lactose [19]. The powder formulation is then administered directly to children or solutions are prepared before use by dissolving these powders in water or syrups [20]. However, there is a risk of insufficient stability of such dosage forms, incomplete dissolution of the active pharmaceutical ingredient and, as a result, unpredictable pharmacodynamics [21].

In this study, the drug was manufactured extemporaneously directly from the substance propranolol hydrochloride, which meets the requirements of pharmacopoeias and regulatory authorities for introduction into small-scale production. Under such conditions, analytical control of impurities and possible degradation products is a necessary component of assessing the quality and safety of the drug for pediatric use throughout the shelf life of the drug.

According to the literature, the most used chromatographic methods for the determination of propranolol hydrochloride and its impurities in medicinal products are high-performance thin-layer chromatography [22] and liquid chromatography [23–25]. Given the small-batch nature of the production of the studied medicinal product, the approach of optimizing the pharmacopoeial methodology of the United States Pharmacopeia mono-

graph Propranolol Hydrochloride Injection [13] was chosen in the work, which ensures regulatory acceptability and simplifies its implementation in practice.

Unlike classical analytical tasks, where pharmacopoeial methods are focused on standardized dosage forms, the analysis of the studied medicinal product was complicated by the presence of excipients. In particular, the "Vanilla" flavoring became a critical factor, since its signal overlapped with the peaks of one of the impurities of propranolol. This necessitated the development of a corrective approach to the processing of chromatographic data to obtain reliable results.

The reliability and reproducibility of the proposed method were confirmed by a comprehensive assessment of the parameters: suitability of the chromatographic system, specificity, linearity, accuracy, precision and limit of quantification. Of particular importance is the confirmation of the reporting limit at a level of no more than 0.1%, which meets the requirements of pharmacopoeias and guarantees the detection of even minimal amounts of impurities that may be therapeutically significant in pediatric practice.

Within the framework of this study, the identified impurity A was selected as a marker impurity in accordance with the requirements of pharmacopoeias, while other possible impurities were considered as unspecified and evaluated in total according to the established acceptance criteria. Thus, the proposed methodology provides control of both identified and unspecified impurities in the composition of the investigated medicinal product.

Stability studies of analytical solutions have proven their ability to be used for 24 hours without loss of analytical accuracy. This is practically important for routine control, as it allows laboratories to work with prepared samples during the working day without the risk of obtaining false results.

Thus, the advantages of the proposed optimized method are the possibility of its application for the control of impurities in the multicomponent drug "Propranolol hydrochloride, 4.28 mg/ml oral solution" for routine analysis without the need to develop alternative analytical approaches. This provides both a scientific and practical basis for harmonizing national quality control standards with international approaches.

Practical significance. The verified liquid chromatography technique can be used in routine quality control of small-batch liquid dosage forms of propranolol hydrochloride for pediatric use to ensure their quality, safety, and stability.

Study limitations. The verification of the liquid chromatography method was mainly concerned with the determination of specific impurity A and non-specific impurities. Other potential degradation products of propranolol require further study in the process of studying the stability of the drug.

Prospects for further research. The next stage of research is planned to develop/verify methods for the identification and quantification of propranolol hydrochloride in a medicinal product for oral use in pediatrics.

6. Conclusions

1. The liquid chromatography technique is characterized by sufficient specificity, linearity in the range of application, as well as confirmed precision and accuracy, which ensures the reliability of results when determining impurities.

2. When conducting the control, the area of the impurity from the "Vanilla" flavoring should be subtracted from the area of impurity A of the test solution. No other negative effects on specificity were detected.

3. The sensitivity of the method meets pharmacopoeial requirements, as the reporting limit is confirmed at ≤ 0.1%.

4. Stability studies have shown that analytical solutions remain stable for 24 hours when stored in the autosampler thermostat.

5. Considering the results presented, the method meets the established validation criteria and can be used to determine the content of related substances in the medicinal product "Propranolol hydrochloride, 4.28 mg/ml oral solution".

Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be made available on reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Authors' contributions

Olena Bevz: Methodology, Investigation, Formal analysis, Validation, Writing – original draft; Anastasiia Slotina: Investigation, Methodology, Visualization, Resources, Funding acquisition, Software; **Olga Kryvanych:** Investigation, Formal analysis, Writing – original draft, Funding acquisition; **Dmytro Soldatov:** Investigation, Visualization, Software, Resources, Funding acquisition; **Nataliia Bevz:** Conceptualization, Methodology, Formal analysis, Validation, Resources, Project administration, Writing – review & editing; **Oleksandr Shmalko:** Methodology, Formal analysis, Visualization, Resources, Funding acquisition; **Victoriya Georgiyants:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision.

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